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HIV-1 differentially modulates autophagy in neurons and astrocytes

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ABSTRACT

Autophagy, a lysosomal degradative pathway that maintains cellular homeostasis, has emerged as an innate immune defense against pathogens. The role of autophagy in the deregulated HIV-infected central nervous system (CNS) is unclear. We have found that HIV-1-induced neuro-glial (neurons and astrocytes) damage involves modulation of the autophagy pathway. Neuro-glial stress induced by HIV-1 led to biochemical and morphological dysfunctions. X4 HIV-1 produced neuro-glial toxicity coupled with suppression of autophagy, while R5 HIV-1-induced toxicity was restricted to neurons. Rapamycin, a specific mTOR inhibitor (autophagy inducer) relieved the blockage of the autophagy pathway caused by HIV-1 and resulted in neuro-glial protection. Further understanding of the regulation of autophagy by cytokines and chemokines or other signaling events may lead to recognition of therapeutic targets for neurodegenerative diseases.

1. Introduction

About 50% of HIV-1-infected patients undergoing long term combination antiretroviral treatment (cART) develop minor to mild HIV-1-associated neurocognitive deficits (HAND). HAND are a well-documented problem, however, the underlying mechanism of neuronal dysfunction is still unclear, but blamed for longer survival of treated HIV-infected patients with residual viral activity and antiretroviral drugs (Sacktor, 2002; Ellis et al., 2007). During HIV-1 infection, the central nervous system (CNS) confronts sustained infiltration of lymphocytes, monocytes, and infected macrophages, as well as neuroinflammatory responses from microglia and macrophages (Hagberg et al., 1988; Budka, 1989; Giulian et al., 1990; Merrill and Chen, 1991; Wahl et al., 1991; Gonzalez et al., 2000; Suh et al., 2014). Upon blood–brain barrier (BBB) compromise in viral infection, damage to neuro-glial cells is anticipated to illicit confounding effects (Dallasta et al., 1999; Xiong et al., 2000; Andersson et al., 2001). As a consequence of postmitotic block, neurons and astrocytes remain throughout the life of an individual. Astrocytes, the predominant cell type in the brain, provide structural and biochemical support to neurons; they are also important in HIV neuropathogenesis (Dong and Benveniste, 2001; Kaul et al., 2001; Mehla et al., 2012). Upon injury or insult, astrocytes are reactivated and provoke a proinflammatory response. HIV-1 exerts direct and indirect effects on neuro-glial cells by diverse mechanisms, leading to oxidative stress and neuronal dysfunctions (Bezzi et al., 2001; Kaul et al., 2001; Chauhan et al., 2003; Turchan et al., 2003).

Macrophages and microglia, either activated or HIV-1-infected, initiate an inflammatory response, further inducing cytokines and chemokines (Bergamini et al., 2000; Mehla et al., 2012), as well as other neurotoxic tryptophan metabolites such as quinolinic acid, arachidonic acid, and nitric oxide or superoxides (Sei et al., 1995; Xiong et al., 2000; Mollace et al., 2001). Synapto-dendritic alteration is indicated in HAND (Ellis et al., 2007). The membrane-associated guanylate kinase protein, post-synaptic density-95 (PSD-95) regulates synaptic activity (Porras et al., 2012; Parsons et al., 2014) and is thought to be linked to the microtubule, actin and dynein proteins which are involved in spine formation (Kuriu et al., 2006). PSD-95 is involved in regulating the nitric oxide signaling pathway, promoting synaptogenesis. Dysregulation in PSD-95 expression is indicated in neurodegeneration (Nikonenko et al., 2008; Fossati et al., 2015). Abnormalities in protein degradation because of autophagy block may lead to PSD-95 accumulation resulting in loss of dendritic spines. This issue needs further investigations.

Autophagy or type 2 programmed cell death, involves the turnover of organelles and proteins to maintain cellular homeostasis at times of stress or starvation (Levine, 2005; Ogata et al., 2006; Gump et al., 2014). Consequently, autophagy is considered to be a prosurvival mechanism and an innate defense against pathogens (Levine and Deretic, 2007; Kundu and Thompson, 2008; Delgado and Deretic, 2009; Delgado et al., 2009; Deretic, 2009). Recently, diverse roles of autophagy has been suggested in innate and adaptive immunity including immune activation and degradation of pathogens which promote the survival of infected cells (Orvedahl and Levine, 2009; Konno et al., 2013; Ma et al., 2013). Thus, the regulation of autophagy is an attractive target for therapeutic intervention in viral infections (Qu et al., 2003; Dengjel et al., 2005; Paludan et al., 2005; Ravindran et al., 2014). Autophagy

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pathway is a multi-step process (Lee, 2009) initiated by the formation of a double-layered membrane called autophagosomes, which are formed by a unique conjugation system. Beclin-1 (BECN-1), one of components of that system, is a mammalian orthologue of yeast Atg6 that localizes to the trans-Golgi network (TGN), mitochondria, and the endoplasmic reticulum as a subunit of mammalian class III phosphatidylinositol 3-kinase (PI3K) and leads to autophagosome formation (Liang et al., 1998; Inbal et al., 2002). After initiation of the autophagosome membrane, a product of the autophagy-related gene 5 (ATG5), in conjugation with ATG12, helps in the formation and elongation of the autophagic isolation membrane (phagophore) (Xie and Klionsky, 2007; Yoshimori and Noda, 2008). ATG5–ATG12 conjugate a key regulator of autophagy is important in the innate anti-viral immune response (Jounai et al., 2007). In addition, ATG5 enhances susceptibility to apoptosis (Yousefi et al., 2006). Thus, BECN-1 and ATG5 are required at the initial step of autophagosome formation. At the final step of membrane formation, microtubule-associated protein (MAP) light chain 3 (LC3), a human analogue of yeast Apg8/Aut7/Cvt5 (ATG8), is cleaved by cysteine proteases to produce LC3-I (18 kDa), located in the cytosolic fraction as soluble protein. Further, LC3-I is cleaved into LC3-II (16 kDa) through the actions of E1- and E2-like enzymes and undergoes post-translational modification by covalent attachment to phosphatidylethanolamine on its C-terminus, leading to its translocation and tight binding to the nascent autophagosomal membrane. Therefore, LC3-II is considered a functional form of LC3; it is used as a specific and reliable marker of autophagy (Qu et al., 2003; Kabeya et al., 2004; Mizushima, 2004; Kouno et al., 2005; Zeng et al., 2006). After fusion with lysosomes, autophagosomes mature into autolysosomes and are destined for degradation, clearing the bulk of cytoplasmic proteins.

Autophagy has been shown to facilitate HIV-1-induced T-cell death via CXCR4 (Espert et al., 2006). BECN-1 serves as a rheostat to control the levels of cellular autophagy and cell death (Levine and Yuan, 2005). During HIV-1 infection in CD4+ T cells, BECN-1 expression and the formation of autophagic vacuoles are significantly reduced, but can be rescued by inducing autophagy by starvation (Zhou and Spector, 2008). Deregulation in autophagy machinery is an important cause of various neurodegenerative diseases (Nixon et al., 2005; Rubinsztein et al., 2005; Yu et al., 2005; Chu, 2006; Zhu et al., 2007; Boland et al., 2008), however, its role in the HIV-1-infected brain needs investigation to decipher the mechanism of toxicity in neurons and astrocytes. In earlier studies, HIV-1 infection of the CNS has shown modulation of the autophagy pathway (Alirezaei et al., 2008; Zhu et al., 2009; Zhou et al., 2011; Fields et al., 2013). However, the mechanism in cells other than neurons, such as astrocytes still needs to be identified. For this reason, we studied the role of autophagy in HIV-1-induced neuro-glial toxicity. Our results showed that HIV-1-induced neuro-glial toxicity was mediated via modulation of autophagy pathway.

2. Materials and methods

2.1. Ethics statement

At the University of Washington, Seattle, human fetal tissues were obtained following written approval from adult female patients undergoing therapeutic abortion at 10–14 weeks gestational age (IRB approval #11449). The use of human fetal tissue was approved by the University of South Carolina (USCeRA#:HSA4636) and is IRB-exempt (45 CFR 46.102(d)). Peripheral blood mononuclear cells (PBMCs) were obtained from the New York Blood Bank (IRB exempt). All cell cultures (primary human and cell lines), HIV-1 infection, and all HIV-1 plasmid DNA studies were done according to university guidelines in a biocontainment facility approved by the Institutional Biosafety Committee (IBC) of the University of South Carolina.

2.2. Reagents

Dulbecco modified minimum essential medium (DMEM), RPMI 1640 and Opti-MEM were purchased from Invitrogen. Bafilomycin-A, 3-methyl adenine (3-MA), rapamycin (Rapa), leupeptin (Leup), and AMD3100 octahydrochloride (AMD) were obtained from Sigma (St. Louis, MO). PE-conjugated anti-CXCR4 was purchased from eBioscience (San Diego, CA) and PE-conjugated IgG1 k isotype control antibodies were purchased from BD Biosciences (San Diego, CA). Mouse IgG_1 isotype control antibodies were purchased from R&D Systems (Minneapolis, MN, USA). Human recombinant soluble CD4 was purchased from Leinco Technologies (St. Louis, MO). Monoclonal antibodies against microtubule-associated protein-2 (MAP-2) and glial fibrillary acid protein (GFAP) were purchased from Sigma-Aldrich (Saint Louis, MO). An antibody to post-synaptic density protein-95 (PSD-95) was obtained from NeuroMab (UC-Davis and Antibodies Inc., CA). Antibodies against BECN1 and ATG12–ATG5 were purchased from Cell Signaling Technologies (Beverly, MA). Monoclonal antibody against LC3 was purchased from MBL Co. (Naka-ku Nagoya, Japan).

2.3. Primary human brain cultures, macrophages, and cell lines

Aborted human fetal brains at 8–14 weeks of gestational age were obtained from the University of Washington and cultured as described earlier (Chauhan et al., 2003; Mehla et al., 2012). Briefly, meninges and blood vessels were removed from the brain cortex and washed twice with Opti-MEM containing 1% penicillin–streptomycin (GibcoBRL). Tissue was mechanically disrupted by passing it once through a 20-mL syringe without a needle. Human fetal astrocytes (HFA) were cultured in DMEM supplemented with 10% FCS and antibiotics. Neuronal cultures were established in Opti-MEM supplemented with 5% FCS, 0.2% N2 supplement (GibcoBRL) and antibiotics. Cultures of primary human fetal neurons (HFN) and astrocytes (HFA) were maintained for at least a month before use in experiments. Astrocytes were used at the 2nd or 3rd passage in order to discourage the growth of contaminating microglia; neurons were used directly from the parent mixed brain cultures. SVGA cells (human astrocytic cell sub-clone of SVG cells, a gift from Dr. Eugene Major, NIH) (Chauhan et al., 2003), were derived from a mixed culture of human fetal glial cells after immortalization with simian virus 40 (SV 40) (Major et al., 1985), and maintained in DMEM supplemented with 2 mM L-glutamine, 10% FBS, and antibiotic solution.

Peripheral blood mononuclear cells (PBMCs) obtained from the New York Blood Bank were separated from 1:2 diluted blood on Histopaque solution (Sigma) in 50 mL sterile tubes. PBMCs obtained from the interface by centrifugation were washed three times with PBS and seeded in 10% FBS and 10% human AB serum (Lonza) containing RPMI. Monocytes were removed by selective adherence to plastic and used as macrophage cultures (Mehla et al., 2012). HEK 293T and Magi cells (HeLa) were obtained through the National Institutes of Health (NIH), AIDS Research and Reference Reagent Program, Division of AIDS, NIAID. HEK 293T cells were maintained in RPMI-1640, and Magi cells in DMEM with 2 mM L-glutamine supplemented with 10% FBS, 1% penicillin, streptomycin, and amphotericin-B (Invitrogen).

2.4. Virus packaging and pseudotyping

X4 HIV-1 NL4-3 (Adachi et al., 1986) and NLENY1 (Kutsch et al., 2002), NL4-3 mutant (D116N) defective in its integrase function (δ-I HIV) (Engelman et al., 1995; Mannioui et al., 2005) and R5 HIV-1 NLENYUV3 (Kutsch et al., 2002), were packaged in 293T cells. Briefly, 17 μg of each of the respective HIV-1 DNA vectors was transfected in 100 mm culture dishes (BD Falcon) using Lipofectamine 2000 (Invitrogen) as described earlier (Vijaykumar et al., 2008; Chauhan et al., 2014). Similarly, HIV-1 NLENY1 was pseudotyped with VSV-G envelope using 4.0 μg of VSV-G expression vector. Lentiviral vector